EXHIBIT A



# Androgen-dependent regulation of human MUC1 mucin expression.

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MUC1 mucin is transcriptionally regulated by estrogen, progesterone, and glucocorticoids. Our objective was to determine whether androgen receptor (AR) activation regulates expression of MUC1. The following breast and prostatic cell lines were phenotyped and grouped according to AR and MUC1 protein expression: 1) AR+MUC1 + [DAR17+19 (AR transfectants of DU-145), ZR-75-1, MDA-MB-453, and T47D]; 2) AR-MUC1 + [DZeo1 (AR-vector control), DU-145, BT20, MDA-MB-231, and MCF7]; 3) AR+MUC1 - (LNCaP and LNCaP-r). Cell proliferation was determined using the MTT assay in the presence of synthetic androgen R1881, 0.1 microM to 1 microM. Cell surface MUC1 expression was determined by flow cytometry in the presence or absence of oestradiol, medroxy progesterone acetate or R1881, with and without 4 hydroxy-flutamide (4-OH), a nonsteroidal AR antagonist. The functional significance of MUC1 expression was investigated with a cell-cell aggregation assay. Only AR+MUC1 + cell lines showed a significant increase in MUC1 expression with AR activation (P (range) =.01 to .0001), reversed in the presence of 4-OHF. Cell proliferation was unaffected. Increased expression of MUC1 was associated with a significant (P (range) = .002 to .001) reduction in cell-cell adhesion. To our knowledge, this is the first description of androgen-dependent regulation of MUC1 mucin. This is also functionally associated with decreased cell-cell adhesion, a recognised feature of progressive malignancy. These findings have important implications for physiological and pathological processes.

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